UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

September 3, 2008

MEMORANDUM

Subject: Efficacy Review for B-Cap® 35 Antimicrobial Agent (EPA Reg. No. 72372-1); DP Barcode: D354107.

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
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Thru: Tajah Blackburn, Team Leader
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To: Karen Leavy / Marshall Swindell
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: FMC Corporation
Peroxygens Division
1735 Market Street
Philadelphia, PA 19103

Formulation from the Label:

Active Ingredient	% by wt.
Hydrogen Peroxide	35 %
Inert Ingredients:	65 %
Total	100 %

I. BACKGROUND

The product, B-Cap 35 Antimicrobial Agent (EPA Reg. No. 72372-1), is an EPA-approved microbiocide for use in controlling slime and sulfate-forming bacteria in process waters, air washing systems, recirculating and once through water cooling towers and systems, and packaging and storage vessels. The product is for industrial use only. The applicant requested that EPA amend the product's registration to include a claim for effectiveness as a sterilant. Previously submitted studies (MRID Nos. 469173-01 and 469173-02) were uncompleted but accepted. Complementary studies were conducted at Reading Scientific Services Limited, The Lord Zuckerman Research Centre, located at Pepper Lane, Reading, RG2 6LA, United Kingdom; and Wickham Laboratories Limited, located on Winchester Road in Wickham, Fareham, Hampshire, PO17 5EU, United Kingdom.

This data package contained a letter from the applicant's representative to EPA (dated May 12, 2008), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), two studies (MRID 474394-01 and 474249-01), Statements of No Data Confidentiality Claims for both studies, a copy of the User Manual for the Clarus Hydrogen Peroxide Vapour Generator, and the proposed label.

Note: The laboratory reports describe studies conducted for the product, FMC Durox LR 35% Hydrogen Peroxide and FMC 35% Durox LR Hydrogen Peroxide. The letter from the applicant's representative to EPA (dated May 12, 2008) states that "Durox LR" is the proposed alternate brand name for "B-Cap 35 Antimicrobial Agent," which is the subject of this efficacy report.

II. USE DIRECTIONS

The product is a sterilant for use in conjunction with the Bioquell Clarus Hydrogen Peroxide Vapor Generator. The hydrogen peroxide vapor is intended for use as a sterilant in enclosures up to 35 cubic feet. Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Ensure that all surfaces are visibly clean and free from gross organic contamination. Connect the Clarus generator to the enclosure. Add the product to the generator according to the operating manual instructions. Seal the enclosure to be sterilized. Apply the product at an injection rate of 3.1 g/minute for 55 minutes. Allow vapor to remain for a minimum of 3 hours. Aerate the chamber until the concentration of hydrogen peroxide vapor is at or below 1.0 ppm.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sterilizers: The AOAC Sporicidal Test is required for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different product lots, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use. Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims

must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice (other than the laboratory that developed the original data). The following minimal confirmatory data must be developed on one sample of the product: Thirty carriers with each of the 2 types of surfaces (silk suture loops and porcelain penicylinders) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Test. These Agency standards are presented in DIS/TSS-9.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 474394-01 "Evaluation of Bioquell "Clarus" Hydrogen Peroxide Vapour Generator in conjunction with FMC Durox LR 35% Hydrogen Peroxide against a method based on the EPA (AOAC) Sporicidal Activity Test," by Gregory Snowdon. Study conducted at Reading Scientific Services Limited. Study completion date – May 2, 2008. Project Number P7-09872.

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). The microorganisms used in the study were obtained from Presque Isle Cultures, Presque Isle, PA as carriers (stainless steel penicylinders and suture loops) inoculated with a certified spore population. One lot (Sample 3; manufactured on October 30, 2007) of the product, FMC Durox LR 35% Hydrogen Peroxide, was tested using a modified version of the "EPA/OPP Microbiology Laboratory ESC, Ft. Meade, MD Standard Operating Procedure for AOAC Sporicidal Activity Test (*Bacillus* species) SOP Number: MB-15-01, Revised: 11-30-06," which is based on the AOAC Sporicidal Activity of Disinfectants Method (Method 966.04). The product lot tested was not at least 60-days old at the time of testing. The product was received ready-to-use.

Sixty (60) penicylinders and sixty (60) suture loops inoculated with *Bacillus subtilis* and sixty (60) penicylinders and sixty (60) suture loops inoculated with *Clostridium sporogenes* were placed in a 1 m^3 test chamber. The test chamber consisted of a "Pharmaflow" isolator joined to a "TPC Microflow" isolator via an interface. Each isolator was connected to a Clarus C Hydrogen Peroxide Vapour Generator. Four metal wire hangers were placed at 60 evenly distributed locations around the "Pharmaflow" isolator. A biological indicator of each type was placed at each of the 60 locations. The neutralization and growth media were placed within the two isolators prior to the start of the test. The sterilization cycle was then started. The interior of the test chamber was conditioned to achieve 40% relative humidity (conditioning phase). Next, the Clarus unit prepared to vaporize the hydrogen peroxide (pre-gassing phase). The Clarus unit injected the vaporized hydrogen peroxide at 3.1 g per minute for 55 minutes (gassing phase). The Clarus unit circulated the product around the test chamber for 3 hours (dwell phase). The Clarus unit removed the hydrogen peroxide from the test chamber by means of a catalyst, until the hydrogen peroxide concentration was less than 1 ppm as determined by Dräger tube (aeration phase). Critical parameters (results not provided) were confirmed by electronic printouts generated by the Clarus units. On completion of the sterilization cycle, the 240 biological indicators were transferred into individual tubes containing neutralization media. This transfer occurred inside the test chamber. Once all 240 biological indicators were placed into the neutralization media, the biological indicators were immediately transferred into individual tubes of secondary growth media. *Bacillus subtilis* carriers were placed into Tryptone Soya Broth and *Clostridium sporogenes* carriers were placed into Thioglycollate with Resazurin. On completion of the transfers, the isolators were opened. The subcultures were removed and transferred to the laboratory within 5 hours. The subcultures were incubated for 21 days at

37°C. All subcultures were checked for the presence of growth after 7 and 21 days. Subcultures were heat shocked for 20 minutes at 80°C, and re-incubated for an additional 72 hours. The subcultures were checked again for the presence of growth. Controls included those for carrier enumeration; neutralization; positive and negative controls; and confirmation of the hydrogen peroxide concentration of the product. Three positive media controls for each carrier type were set up per test run (i.e., tubes of each media type containing a single biological indicator). Three negative media controls were set up per test run (i.e., unopened tubes of Tryptone Soya Broth and Thioglycolate with Resazurin).

Note: The study was conducted according to GLP standards with the following exceptions: "1. The site at Bioquell has not been inspected by GLP-MA. 2. Not all pieces of equipment at the Bioquell site have been qualified." The Clarus Vapour Generators have not been formally qualified; however, the generators have been operationally qualified by annual calibrations and method specific titrations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 474249-01 "Evaluation of Bioquell Clarus Hydrogen Peroxide Vapour Generator Using FMC 35% Durox LR Hydrogen Peroxide Sample 6 Manufactured 4th September 2007 as Decontamination Agent By Modified AOAC Sporicidal Activity Test Method 966.04 In Accordance With EPA Requirements," by Susan Wood. Study conducted at Wickham Laboratories Limited. Study completion date – March 31, 2008. Study Number 1470 (Project CN00115439b).

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). The microorganisms used in the study were obtained from Presque Isle Cultures, Erie, PA as carriers (stainless steel penicylinders and polyester suture loops) inoculated with a certified spore population. One lot (Sample 6; manufactured on September 4, 2007) of the product, FMC 35% Durox LR Hydrogen Peroxide, was tested using a modified version of the "EPA/OPP Microbiology Laboratory ESC, Ft. Meade, MD Standard Operating Procedure for AOAC Sporicidal Activity Test (*Bacillus* species) SOP Number: MB-15-01, Revised: 11-30-06," which is based on the AOAC Sporicidal Activity of Disinfectants Method (Method 966.04). The product lot tested was at least 60-days old at the time of testing. The product was received ready-to-use.

Thirty (30) penicylinders and thirty (30) suture loops inoculated with *Bacillus subtilis* and thirty (30) penicylinders and thirty (30) suture loops inoculated with *Clostridium sporogenes* were placed in a 1 m^3 test chamber. The test chamber consisted of a four-glove "Pharmaflow" isolator joined to a two-glove "TPC Microflow" isolator via a lockable interface. Each isolator was connected to a Clarus Hydrogen Peroxide Vapour Generator. Four metal wire hangers were placed at 30 locations within the "Pharmaflow" isolator. A biological indicator of each type was placed at each of the 30 locations. The neutralization and growth media were placed within the two isolators prior to the start of the test. The sterilization cycle was then started. The interior of the test chamber was conditioned to achieve 40% relative humidity (conditioning phase). Next, the Clarus unit prepared to vaporize the hydrogen peroxide (pre-gassing phase). The Clarus unit injected the vaporized hydrogen peroxide at 3.1 g per minute for 55 minutes (gassing phase). The Clarus unit circulated the product around the test chamber for 180 minutes (i.e., 3 hours) (dwell phase). The Clarus unit removed the hydrogen peroxide from the test chamber by means of a catalyst, until the hydrogen peroxide concentration was less than 2 ppm as

determined by Dräger tube (aeration phase). Critical parameters (results not provided) were confirmed by electronic printouts generated by the Clarus units. On completion of the sterilization cycle, the 120 inoculated biological indicators were transferred into individual tubes containing neutralization media. The biological indicators were transferred to the growth media of the same type. *Bacillus subtilis* carriers were placed into Tryptone Soya Broth and *Clostridium sporogenes* carriers were placed into Fluid Thioglycollate Medium USP. This transfer occurred inside the test chamber. On completion of the transfers, the isolators were opened. The subcultures were removed and transferred to the laboratory within 5 hours. The subcultures were incubated for 21 days at 37°C (or until growth was seen). All subcultures were checked daily for the presence of growth. Subcultures showing no growth after 21 days were heat shocked for 20 minutes at 80°C, and re-incubated for an additional 72 hours. The subcultures were checked again for the presence of growth. Controls included those for carrier enumeration; neutralization; viability (i.e., positive control); sterility (i.e., negative broth control); confirmation of the hydrogen peroxide concentration of the product; and acid resistance of spores.

Note: The study was conducted according to GLP standards with the following exceptions: "1. The site at Bioquell has not been inspected by GLP-MA; 2. The Clarus Vapour Generators have not been formally qualified. The Clarus Vapour Generators have been operationally qualified by annual calibrations and method specific titrations to demonstrate fitness for purpose and use."

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID Number	Organism	Carrier Type	No. Exhibiting Growth / Total No. Tested		Biological Indicator Count (CFU/Carrier)
			Sample 3	Sample 6	
474394-01	*Bacillus subtilis*	sutures	0/60	---	1.4×10^6
		penicylinders	0/60	---	2.70×10^5
474394-01	*Clostridium sporogenes*	sutures	0/60	---	3.36×10^5
		penicylinders	0/60	---	4.94×10^5
474249-01	*Bacillus subtilis*	sutures	---	0/30	1.3×10^6
		penicylinders	---	0/30	1.1×10^6
474249-01	*Clostridium sporogenes*	sutures	---	0/30	1.2×10^6
		penicylinders	---	0/30	1.6×10^6

VI. CONCLUSIONS

1. The submitted efficacy data (MRID 474394-01 and 474249-01) in conjunction with previously submitted efficacy data (MRID Nos. 469173-01 and 469173-02) **support** the use of the product, B-Cap 35 Antimicrobial Agent (also known as "Durox LR" or Durox LR Hydrogen Peroxide), when used in conjunction with the Clarus Hydrogen Peroxide Vapour Generator, as a sterilant against *Bacillus subtilis* and *Clostridium sporogenes.* Conditions of the application were as follows: product injection rate = 3.1 g/minute for 55 minutes; exposure time of 3 hours; 1 m^3 (i.e., 35 ft^3) enclosure.

One product lot tested was at least 60 days old at the time of testing. Biological indicator counts were at least 2 x 10^5 CFU/carrier, which is the average spore count recommended in EPA SOP MB-15-00. *Clostridium sporogenes* test spores (suture loops) showed resistance to acid for >2 minutes, in both primary and secondary subcultures. *Clostridium sporogenes* test spores (penicylinders) showed resistance to acid for >2 minutes, in secondary subcultures. *Bacillus subtilis* test spores (penicylinders and suture loops) showed resistance to acid for >2 minutes, in both primary and secondary subcultures. Neutralization testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, B-Cap® 35 Antimicrobial Agent is an effective sterilant, when used in conjunction with the Clarus Hydrogen Peroxide Vapour Generator, for treating enclosures up to 35 ft^3. A product injection rate of 3.1 g/minute for 55 minutes and exposure time of 3 hours are specified. **Data provided by the applicant support these claims.**

2. The applicant must make the following changes to the proposed label, as appropriate:

- Under the "Storage and Disposal" section of the proposed label, change "Cap 35 Antimicrobial Agent" to read "B-Cap 35 Antimicrobial Agent.

- Under the "Air Washers ..." section of the proposed label, change "Once control is achieved continuous feed of" to read "Once control is achieved **use a** continuous feed of."

- In the "Control of Slime ..." heading of the proposed label, change "**other** Microorganism" to read "**Other** Microorganisms."

- On page 2 of the product label under the directions for using the product as a sterilant, change (in numerous locations) "**Operating Manual**" to read "**User Manual.**"

- On page 2 of the product label under the directions for using the product as a sterilant, **identify the types of surfaces/objects that may be sterilized**.